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TECHNICAL MANUSCRIPT 591

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Kiyoshi Higuchi

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DEPARTMENT OF THE ARMY
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Frederick, Maryland

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Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B562602AD01

February 1970

REQUIREMENTS FOR CHOLESTEROL, HEMATIN, AND LECITHIN FOR OPTIMAL GROWTH OF A PORCINE KIDNEY CELL LINE*

ABSTRACT

The nutritional requirements for growth of a porcine kidney (PK) cell line in a chemically defined medium were studied in cells grown as monolayer cultures in T-30 Falcon plastic flasks incubated at 36 C with caps tightened. The PK cell appeared to be unique among a variety of heteroploid cell lines in its requirement for a number of unusual substances. Successful propagation of PK cells was obtained in a serum-free defined medium that contained cholesterol (2×10^{-5} M), hematin (0.5 μg/ml), lecithin (2 μg/ml), and coenzyme Q_{10} (1 μg/ml). The PK cell line may serve as a useful tool in a study of intermediary lipid metabolism at the cellular level.

Bailey[1] showed that several established mammalian cell lines were capable during growth of synthesizing cellular lipids from simple precursors such as glucose and acetate. Radioactively labeled precursor carbon was recovered in cholesterol, triglycerides, and phospholipids. Bailey also showed that these cells were able to utilize lipids supplied exogenously when placed in a growth medium containing serum. On the other hand, Sato and co-workers[2] reported that cholesterol was required at a concentration of 1 μg/ml for efficient cloning of HeLa S3 cells in a medium containing highly dialyzed serum. Lockart and Eagle,[3] however, found that cholesterol did not enhance the growth of isolated single HeLa cells in a similar experiment. They attributed the contradictory results to differences in methods of inoculum preparation. An alternative explanation that can be considered is that differences in degree of dialysis of serum caused failure to demonstrate a need for cholesterol. Cloning presents exacting conditions with respect to nutritional requirements; it is therefore not surprising that a requirement for cholesterol by continuous cell lines had been detected previously only in cloning experiments.

The above results indicate that a requirement for cholesterol could be demonstrated only under special conditions. Recently, however, Holmes and co-workers[4] reported that human diploid cell cultures grew only if cholesterol was supplied at levels of 1 mg/liter in a defined medium fortified with purified serum fractions. These workers suggested that a requirement for cholesterol was characteristic of primary diploid mammalian cells and implied that heteroploid cells do not require the substance in their growth medium.

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Work in our laboratory has been devoted to studies on the cultivation of established cell lines in various chemically defined media. A variety of cell lines has been grown successfully in a rather simple medium whose composition is shown in Table 1. Our results, therefore, were in accord with the hypothesis of Holmes and co-workers. During the past year, however, a porcine kidney (PK) line described by Inoue and Ogura[5] failed to grow in our basal defined medium. A variety of substances were tested in order to promote growth of PK cells; among these, cholesterol, lecithin, hematin, and coenzyme Q_{10} appeared to improve growth significantly. A combination of these substances when added to the basal medium permitted indefinite serial passages of PK cells, even with inoculum as low as 20,000 cells/ml.

TABLE 1. COMPOSITION OF THE CHEMICALLY DEFINED MEDIUM

L amino acids	mg/liter	Vitamins	mg/liter
arginine·HCl	32	D-biotin	1.0
asparagine·H_2O	150	choline Cl	1.0
cysteine HCl·H_2O	22	D-Ca-pantothenate	2.0
glutamine	196	folic acid	1.0
histidine·HCl·H_2O	63	myo-inositol	1.0
isoleucine	33	pyridoxal HCl	1.0
leucine	26	riboflavin	0.1
lysine·HCl	28	thiamin HCl	1.0
methionine	15	vitamin B_{12}	0.002
phenylalanine	33	nicotinamide	1.0
proline	115		
serine	105	Salts	
threonine	12		
tryptophan	6.3	NaCl	7400
tyrosine	46	KCl	400
valine	35	$NaH_2PO_4·H_2O$	100
		$NaHCO_3$	670
Misc. substances		$CaCl_2·2H_2O$	148
		$MgCl_2·6H_2O$	305
glucose	1800	Na-pyruvate	110
gluconic acid	178	$FeNH_4(SO_4)_2·12H_2O$	4.85
methylcellulose (15 cps grade)	500	$ZnSO_4·7H_2O$	0.288
phenol red	10		
insulin (lente Iletin)	(0.05 U/ml)		
penicillin G-sodium	67		
streptomycin SO_4	100		

The growth response of PK cells to graded levels of cholesterol in a medium containing coenzyme Q_{10} but no hematin or lecithin is shown by data plotted in Figure 1. Cultures were inoculated with 62,000 cells per ml in Falcon T-30 plastic flasks and incubated at 36 C for 1 week. Media were replaced twice during the growth cycle. Cell protein values were determined by the method of Oyama and Eagle[6] with crystalline bovine serum albumin as the protein standard. Approximately 1×10^{-5} M cholesterol (corresponding to about 4 μg/ml) produced peak yields of 114 μg of cellular protein per ml of growth medium.

Of the four substances mentioned above that were tested, cholesterol yielded the greatest enhancement of growth of PK cells. The results of single additions of hematin, lecithin, and coenzyme Q_{10} to the basal defined medium containing 2×10^{-5} M cholesterol are presented in Table 2. Both hematin (0.5 μg/ml) and lecithin (2 μg/ml) produced significant improvement in growth of PK cells in a medium containing cholesterol. Coenzyme Q_{10} appeared to have no stimulatory effect in this test; however, in other experiments, this compound seemed to benefit the growth of PK cells.

TABLE 2. EFFECTS OF HEMATIN, LECITHIN, AND COENZYME Q_{10} ON GROWTH OF PORCINE KIDNEY CELL LINE

Test Substance Added to Basal Medium[a]	Cell Protein Yield,[b] μg/ml
None	127, 114 (120)
Hematin (0.5 μg/ml)	232, 265 (298)
Lecithin (2 μg/ml)	242, 237 (240)
Coenzyme Q_{10} (1 μg/ml)	80, 104 (97)
All three (as above)	307, 343 (327)

a. The basal medium contained cholesterol at 2×10^{-5} M.
b. Averages in parentheses.

To our knowledge, the stimulatory effects of hematin and lecithin on growth of cultured animal cells have not been reported previously. Further studies will be made to determine more precisely the concentrations of these substances needed for optimal growth of PK cells.

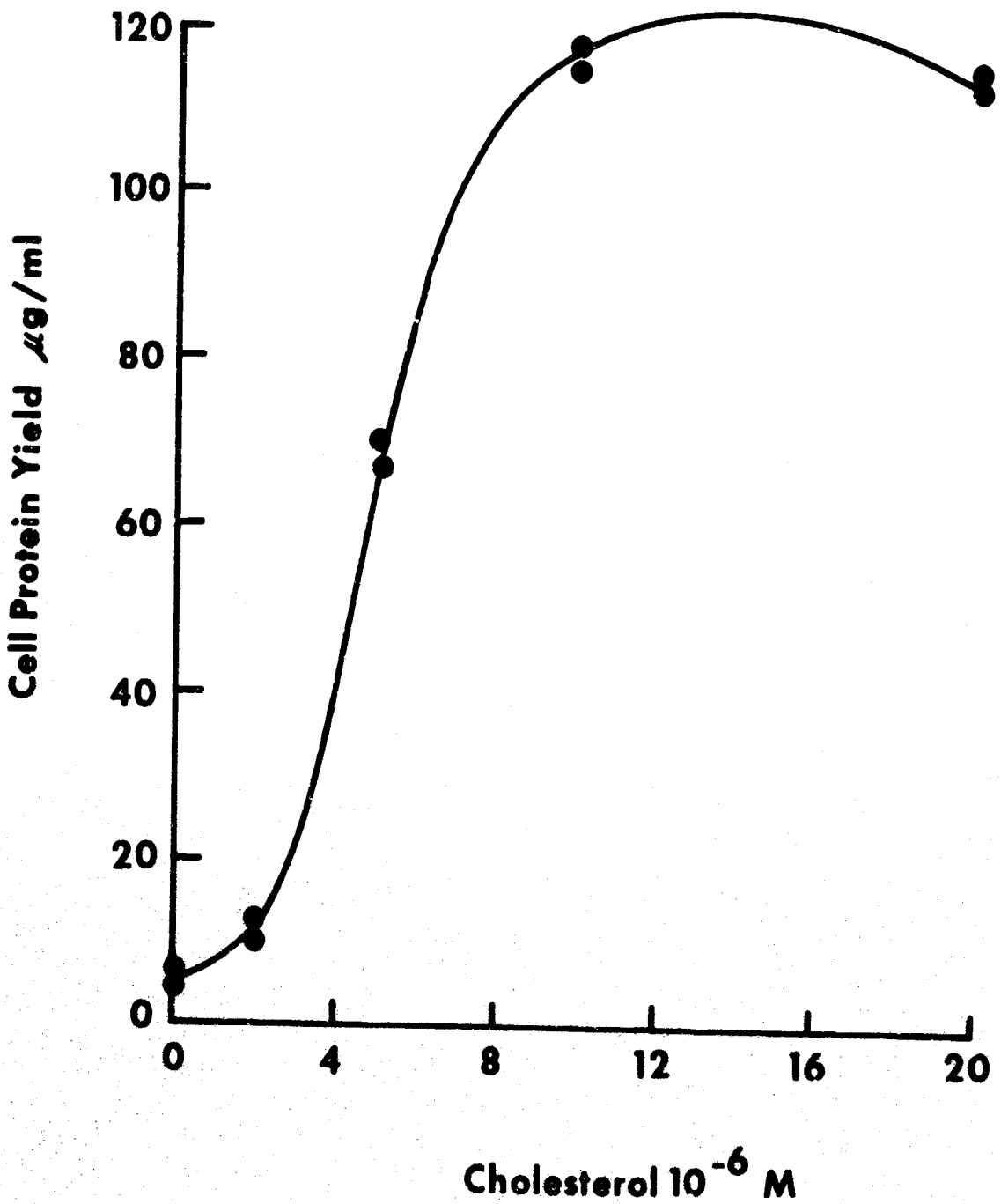

FIGURE 1. Growth Response of Porcine Kidney Cell Line to Cholesterol.

In summary, we have obtained excellent growth of the porcine kidney (PK) cell line described by Inoue and Ogura[6] in a completely chemically defined medium when provided with cholesterol (2×10^{-5} M), hematin (0.5 μg/ml), lecithin (2 μg/ml), and coenzyme Q_{10} (1 μg/ml). The stimulatory effect of coenzyme Q_{10} has been equivocal, but in the presence of all four compounds, more than 13 serial transfers have been made successfully that involved inoculum levels as low as 20,000 cells per ml. The PK cell line was unique among a number of established cell lines of various species of origin in being unable to synthesize adequate levels of lipids for growth. Therefore, it may serve as a useful tool in future research leading to elucidation of mechanisms of intermediary lipid metabolism.

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